MATURATION OF VOMERONASAL RECEPTOR NEURONS IN COCULTURE SYSTEM

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We developed a culture system of vomeronasal (VN) receptor neurons to analize the functional role of pheromonal recognition in virto. The spherical structures surrounding a cavity showed VN organs like features, referred to as a VN pocket. The VN pockets contained both VN receptor neurons without microvilli and supporting cells. In the VN pockets, few olfactory marker protein (OMP) expressing cells were recognized. It has been indicated that the VN receptor neurons in the VN pockets were immature. These VN pockets are not suitable to use for a pheromonal recognition analysis. In the present study, we established a coculture system to induce the maturation of VN receptor neurons. The VN pockets were cocultured with accessory olfactory bulb (AOB) cells, which are targets of VN receptor neurons. At 2weeks coculture, the number of OMP-positive cells was greater in coculture with AOB cells than in VN pocket-alone culture. In these VN pockets, many VN receptor neurons with microvilli were observed. These results indicate that the VN receptor neurons are promoted maturation by use of coculture system. The AOB cells induced maturation of VN receptor neurons in VN pockets.

TECTAL PERIVENTRICULAR NEURONS PROJECTING TO THE TORUS SEMICIRCULARIS IN RAINBOW TROUT

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To identify periventricular neurons projecting from the optic tectum (OT) to the torus semicircularis (TS) in rainbow trout, we examined the fiber connections between the OT and the TS by injection of a tracer (biotinylated dextran amine) into the TS. Labeled periventricular neurons were located in a superficial part of the stratum periventriculare, and they were classified into at least three types. (1) Pyriform neuron with an apical dendrite ramifying at the stratum fibrosum et griseum superficiale. (2) Monopolar neuron with dendritic branches restricted in the stratum album centrale. (3) Pyriform neuron with a long apical dendrite ramifying at the striatum opticum. The morphology of Type 3 neurons resembles that of efferent neurons to the nucleus isthmi in carp, and this type neuron was presumably labeled from passing axons through the TS.

COMPARISON OF EFFECT OF ANESTHETICS ON THE TASTE AVERSION MEMORY BETWEEN MOUSE AND RAT

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Most animals come to avoid the taste, if the visceral malaise accompanies experience of the novel taste. This is called the conditioned taste aversion (CTA). We have already reported that intravenous anesthetics such as propofol suppress CTA in the low concentration but enhance in the high concentration. At present experiment, we compared the results obtained from the rat with those from the mouse, which represents different nature in taste preference and sensitivities to emotional stimulus. We report results obtained from Fos-immunohistochemical study as well as behavioral experiments.

HISTOCHEMICAL PROPERTIES OF THE CEREBELLAR NEURONS IN GOLDFISH

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In the teleosts cerebellum, the eurydendroid cells are considered to project to other brain regions and reportedly are distributed mainly in the Purkinje cell layer. Therefore, there are two neuron types in the Purkinje cell layer in the teleost cerebellum. In the present study, to reveal histochemical features and candidate neurotransmitters of these neurons in the goldfish corpus cerebelli, retrograde labeling and immunohistochemical staining with anti-GABA, anti-aspartate or anti-zebrin II were performed in the same specimen. As a result, no GABA-like and zebrin II immunoreactivity was detected in the retrogradely labeled eurydendroid cells. Retrogradely labeled cells in the granule cell layer were surrounded by the zebrin II-positive fibers. About half of retrogradely labeled eurydendroid cells were immunoreactive to the anti-aspartate antibody. These findings suggest that some eurydendroid cells contain aspartate as a neurotransmitter, send an excitatory stimuli to other brain regions, and possibly receive GABAergic inhibitory inputs from the Purkinje cells.

PROPERTIES OF AMPA-TYPE GLUTAMATE RECEPTORS THAT MEDIATE HETEROSYNAPTIC INHIBITION IN THE RAT CEREBELLAR CORTEX

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We have previously reported that repetitive activation of climbing fibers (CFs) in the cerebellar cortex elicits presynaptic inhibition of GABAergic transmission (that is, disinhibition) from basket cells (BCs) to Purkinje cells (PCs) through activation of AMPA-type glutamate receptors (AMPARs). However, the properties, especially localization and activation mechanism, of AMPARs involved in this presynaptic inhibition are not known. We thus examined receptor mechanisms underlying the disinhibition by using electrophysiological tests with AMPAR-specific pharmacological tools, such as philanthotoxin-433, bromo-homoibotenic acid and γ-D-glutamlyglycine. Results from these pharmacological tests suggest that the CF-induced disinhibition was mainly mediated by Ca^{2+}-impermeable AMPARs, possibly GluR2/GluR3 heteromers expressed at presynaptic terminals of the BC, activated by the excitatory amino acid spilled out of the CF-PC synapses. Such a spillover-dependent heterosynaptic interaction between different synaptic inputs (*i.e.*, excitatory CF and inhibitory BC) converging on the same PC would profoundly influence the activity of the PC, the sole output neuron of the cerebellar cortex.

ACTUAL AND EFFECTIVE UTILIZATION OF NEURON SPECIFIC mRNAs TO IDENTIFY PARTICULAR NEURON GROUPS PARTICIPATING IN PARTICULAR FISH BEHAVIOUR

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We have obtained mRNAs encoding choline acetyltransferase (ChAT) and c-Fos protein from the goldfish (*Carassius auratus*) brain. By using specific probes made from cDNAs corresponding to the mRNAs, *in situ* hybridizations were carried out on sections of the fish brainand spinal cord. Expressions of ChAT mRNA were found in cytoplasm of spinal motoneurons and neurons in the oculomotor and trochlear nuclei. Expression of the *c-fos* mRNA encoding c-Fos protein was induced artificially by two ways; a light-stimulation and a prolonged forced swimming. In the former case, a significantly stronger expression of *c-fos* mRNA was detected in neurons in the optic tectum of the stimulated fish in comparison with the controls. In the latter, the *c-fos* mRNA was expressed in motoneurons in the spinal cord and Nflm neurons in the midbrain tegmentum. We consider that the probe for ChAT mRNA is an useful tool to identify cholinergic neruons at least in cyprinid fish and that the *c-fos* probe could be apply to define some brain centers involving not only in sensory and motor functions, but also in emotion, learning, and other brain functions.

DISTRIBUTION OF PROLACTIN RECEPTOR IN THE NEWT BRAIN

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In the male newts (*Cynops pyrrhogaster*), prolactin (PRL) acts directly on the central nervous system and induces courtship behavior. As a step to elucidate the localization of neurons on which PRL acts, we developed polyclonal antibody against an oligopeptide. Immunohistochemical study of the newt brain was performed using the antibody thus produced. The specificity of this antibody was confirmed by Western blot analysis using membrane protein from COS-7 cells in which newt PRLR was transiently expressed. PRLR-like immunoreactive cells were observed in anterior preoptic area, medial amygdala, posterior preoptic nucleus, suprachiasmatic nucleus, ventral hypothalamic nucleus, nucleus of periventricular organ, and choroid plexus. In addition, we performed in situ hybridization (ISH) by using newt PRLR antisense RNA probe to detect PRLR mRNA in the brain. The specific signals of PRLR mRNA were detected mainly in the anterior preoptic area, posterior preoptic nucleus, and choroid plexus.

ANALYSIS OF MOLECULAR BASIS OF ASSOCIATIVE VISUAL LEARNING IN THE HONEYBEE *APIS MELLIFERA* L.

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We previously established modified associative visual learning protocol of the honeybee (*Apis mellifera* L.), in which the monochromatic light stimulus (620nm) was associated with proboscis extension reflex (PER) to sucrose solution, aiming at identifying the molecular and neural basis underlying visual information processing. The results suggested that the learning capacity differs among colony members. In the present study, to examine the relation between division of labors and learning capability of the workers, we compared the learning capacities of the nurse bees, guard bees and foragers. As a result, there was no significant difference in their learning